The geldanamycin analogue 17-allylamino-17-demethoxygeldanamycin inhibits the growth of GL261 glioma cells *in vitro* and *in vivo*

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Geldanamycin is a naturally occurring benzoquinone ansamycin product of *Streptomyces geldanus* that binds the protein chaperone heat shock protein 90. As geldanamycin binds to heat shock protein 90 interfering with its function and heat shock protein 90 is overexpressed in many cancers, heat shock protein 90 has become a target for cancer therapy. As the geldanamycin analogue 17-allylamino-17-demethoxygeldanamycin has a favorable toxicity profile, it is being tested extensively in clinical trials in patients with advanced cancer. In this study, GL261 glioma cells from C57BL/6 mice were used to investigate the anti-tumor effect of 17-allylamino-17-demethoxygeldanamycin both *in vitro* and *in vivo*. Heat shock protein 90 inhibitors possess potent anti-proliferative activity, usually at low nanomolar ranges, owing to their pharmacological characteristics of binding tightly to heat shock protein 90, coupled with a slow dissociation rate. We found that 17-allylamino-17-demethoxygeldanamycin at doses as low as 200 nmol/l showed anti-tumor activity within 24 h of treatment. Treatment with 17-allylamino-17-demethoxygeldanamycin arrested GL261 cells in the G_2 phase of the cell cycle associated with the downregulation of cyclin B1. Low doses of 17-allylamino-17-demethoxygeldanamycin significantly inhibited migration of GL261 cells within 16 h of treatment, concomitant with the downregulation of phosphorylated focal adhesion kinase and matrix metalloproteinase 2 secretion. Using an orthotopic glioma model with well-established intracranial tumors, 3 weekly cycles of 17-allylamino-17-demethoxygeldanamycin significantly reduced tumor volumes of treated animals compared with untreated controls ($P=0.002$). Given these promising results, clinical testing of 17-allylamino-17-demethoxygeldanamycin or other novel heat shock protein 90 inhibitors being developed should be considered for glioma patients whose tumors remain refractory to most current treatment regimens. *Anti-Cancer Drugs* 18:875–882 © 2007 Lippincott Williams & Wilkins.

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Introduction

Geldanamycin is a naturally occurring benzoquinone ansamycin product of *Streptomyces geldanus* that binds the protein chaperone heat shock protein 90 (Hsp90). Geldanamycin functionally inhibits the activity of several different protein kinases, interferes with signal transduction pathways and inhibits the proliferation of a wide variety of tumor cell lines [1]. We demonstrated previously that geldanamycin has anti-tumor activity in human glioma cells *in vitro* associated with its ability to decrease migration of several glioma lines, decrease phosphorylation of focal adhesion kinase (FAK) and inhibit hypoxia-mediated induction of hypoxia-inducible factor (HIF)-1α [2]. As FAK may have an important role in the invasion of glioma cells and HIF-1α plays an important role in angiogenesis, geldanamycin or one of its analogues may be useful clinically in the treatment of highly invasive and angiogenic gliomas.

As geldanamycin binds to Hsp90, interfering with its function [1], and Hsp90 is overexpressed in many cancers, Hsp90 has become a target for cancer therapy [3–7]. Geldanamycin, however, induces liver toxicity; the less-toxic geldanamycin analogue, 17-allylamino-17-demethoxygeldanamycin (17-AAG) is now being tested extensively in clinical trials in patients with advanced cancer [6]. Anti-tumor activity has been observed in patients with breast cancer, multiple myeloma and other cancers [6]. In this study, GL261 glioma cells from C57BL/6 mice were used to investigate the anti-tumor

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effect of 17-AAG both *in vitro* and *in vivo*. We have developed an experimental murine GL261 animal model that mimics the aggressive and invasive growth observed in human gliomas [8–11]. We show that 17-AAG results in decreased growth of GL261 cells *in vitro* and *in vivo*, and could be considered a potential effective anti-tumor drug for patients with glioma.

Materials

Cells and reagents

The GL261 murine glioma cells were obtained from NCI-Frederick Cancer Research National Tumor Repository (Frederick, Maryland, USA). Cells were cultured in 5% CO_2 and 95% humidified air atmosphere at 37°C in Dulbecco's modified Eagle medium (DMEM) (Gibco BRL, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, Georgia, USA), 0.25% gentamicin and 1% L-glutamine. Cultures were split every 3 days to ensure logarithmic growth. The geldanamycin analogue 17-AAG was provided by the Cancer Treatment and Evaluation Program, National Cancer Institute (Bethesda, Maryland, USA), dissolved in dimethylsulfoxide (DMSO) and the stock (200 µmol/l) was stored at −80°C. Staurosporine (STA; Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in DMSO and the stock (1 mmol/l) was stored at −20°C. Taxol (Sigma-Aldrich) was dissolved in DMSO and the stock (100 µmol/l) was stored at −80°C. Hydroxyurea (HU; Sigma-Aldrich) was prepared fresh in DMEM to a stock concentration of 10 mol/l. *p*-Aminophenylmercuric acetate (APMA; Sigma-Aldrich) was prepared fresh in DMSO to a stock concentration of 10 mg/ml.

Cytotoxicity assay

Cells (5×10^5) were seeded in 10-cm culture dishes (Falcon, Franklin Lakes, New Jersey, USA) for 24 h before treatment. Cells were exposed to various concentrations of 17-AAG (200, 500 and 1000 nmol/l) for 24, 48 or 72 h. Total cells were harvested by collecting nonadherent floating cells combined with trypsinized adherent cells. Viability was assessed using the Trypan blue exclusion assay. Three independent experiments were performed.

Flow cytometry analysis

Total cells were harvested from each culture condition at the appropriate time interval. For cell cycle analysis, cells were stained with propidium iodide (PI) and DNA content was used to distinguish the cell cycle phases as previously described [12]. To detect cells in M phase of the cell cycle, cells were analyzed with the anti-MPM-2 monoclonal antibody (1 µg/ml, #05-368; Upstate Biotechnology, Lake Placid, New York, USA) and stained with PI before analysis [13,14]. Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Bedford, Massachusetts, USA) and histograms were obtained using the ModFit LT software (Verity Software House, Topsham, Maine, USA). Two independent experiments were performed.

Cell migration assay

Cells (2×10^4) were plated onto BD Biocoat chambers (#354578; BD Bioscience Discovery Labware, Bedford, Massachusetts, USA) as previously described [15]. Briefly, cells in 400 µl of DMEM with 10% fetal bovine serum were seeded onto the upper compartment of each insert and placed into wells containing 750 µl of complete medium. Cells were allowed to adhere to the insert for 1.5 h and then medium in the upper chamber was replaced with complete medium with or without 200 nmol/l 17-AAG or with and without 10 mmol/l HU. Following incubation for 16 h at 37°C, to allow cells to migrate through the membrane, the number of migrated cells was quantified. Experiments were performed in duplicate. Data from four independent experiments were pooled for statistical analysis.

Western blot analysis

Cells were untreated or treated with 200 or 500 nmol/l 17-AAG for the indicated time and collected for protein extraction. Western blot analysis was performed for the expression of phospho-FAK (#ab4792; Abcam, Cambridge, Massachusetts, USA), MPM-2 (#05-368; Upstate Biotechnology), cyclin B1 (#sc-245; Santa Cruz Biotechnology) and β-actin (#MAB1501; Chemicon International, Temecula, California, USA) as described previously [15,16].

Gelatin zymography

The matrix metalloproteinase (MMP)-2 released into the conditioned medium was measured by gelatin zymography as described previously [1]. Cells (5×10^5) were seeded in six-well plates in 2 ml complete growth medium for 24 h, then washed and incubated in 750 µl serum-free DMEM for an additional 24 h in the absence or presence of 200 nmol/l 17-AAG. Medium was collected and stored at −80°C until analyzed. To activate pro-MMP-2, AMPA was added to samples of conditioned medium to give a final concentration of 1 mmol/l in the presence or absence of different concentrations of 17-AAG. Samples were incubated for 1 h at 37°C and immediately electrophoresed on 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels. Gels were photographed and densitometrically scanned using NIH Image software. The gelatinase standard (Chemicon International) was used as a positive control. Data from three independent experiments were pooled for statistical analysis.

Intracranial implantation study

Female C57BL/6 mice (10–12 weeks old) were purchased from Taconic (Germantown, New York, USA) and maintained in accordance with the protocol approved by the Institutional Animal Care and Use Committee. Cells

(1×10^5) were implanted stereotactically into the right hemisphere as described previously [10]. On day 7 following implantation, animals were randomly assigned into control and test groups ($N = 3$–4/group). Treatment consisted of intraperitoneal injection of vehicle (10% DMSO/saline) or 17-AAG (50 mg/kg in 10% DMSO/saline) three times a week for up to 28 days. Groups of animals were killed on days 21 and 28. Before killing, the animals were anesthetized and then perfused intracardially with phosphate-buffered saline followed by 4% paraformaldehyde. Brains were removed and sliced into 2-mm coronal sections before processing for paraffin embedding. Tumors in the hematoxylin and eosin-stained coronal sections were measured to determine tumor volumes. Tumor volume was calculated using the formula: $L \times S^2 \times 1/2$, where L is longest tumor diameter and S the shortest tumor diameter.

Statistics

All calculations were performed using GraphPad Prism 3.0 (GraphPad Software, San Diego, California, USA). Results are expressed as mean ± SD. The two-sided *t*-test was used to compare the difference between treatment groups for the migration and MMP-2 assays and the animal studies. Differences were considered significant if the *P*-value is less than 5% ($P < 0.05$).

Results

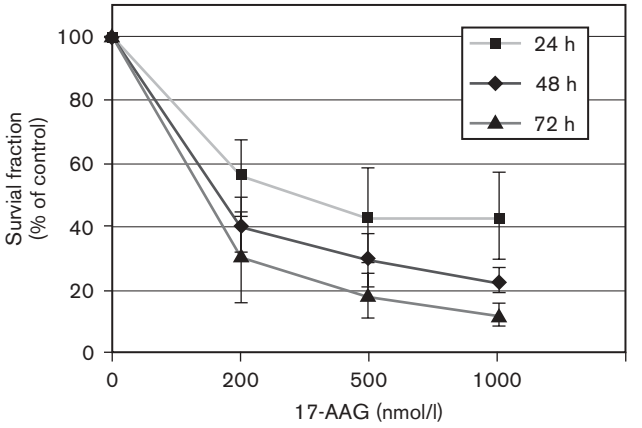
17-allylamino-17-demethoxygeldanamycin inhibits proliferation of GL261 cells

Before examining the anti-tumor effects of 17-AAG *in vivo*, we evaluated its activity on the growth of GL261 cells *in vitro*. Time course experiments were performed to determine the effect of different doses of 17-AAG on the growth of GL261 cells. Data from three independent experiments are summarized. As shown in Fig. 1, the relative cell number in drug-treated cultures decreased in a dose- and time-dependent manner. The dose of 1000 nmol/l 17-AAG inhibited proliferation of GL261 cells by 80% at 48 h compared with 60% after 24 h of exposure to either 500 or 1000 nmol/l treatments. Exposure of GL261 cells to 200 and 500 nmol/l 17-AAG for 24 h, however, decreased overall growth by 50–60%. We selected the doses of 200 and 500 nmol/l for further testing.

17-allylamino-17-demethoxygeldanamycin arrests GL261 in G_2 phase of the cell cycle

Flow cytometry was used to analyze changes in cell cycle distribution of GL261 cells exposed to different doses of 17-AAG and for increasing times of drug exposure. Data from two independent experiments are summarized. As shown in Fig. 2a, the distribution of cells in different phases of the cell cycle showed an increase in the percentage of cells in the G_2/M phase, beginning at 24 h of treatment with 200 nmol/l compared with untreated cells. As the dose increased from 200 to 1000 nmol/l, there was a concomitant increase in the number of cells arrested in G_2/M phase. This redistribution resulted in decreased numbers of cells in S phase and marginally increased numbers of cells in G_0/G_1 following a small decrease in G_0/G_1 observed with 200 nmol/l 17-AAG. This pattern of cell cycle redistribution did not change appreciably over the 72 h interval of treatment.

Fig. 1

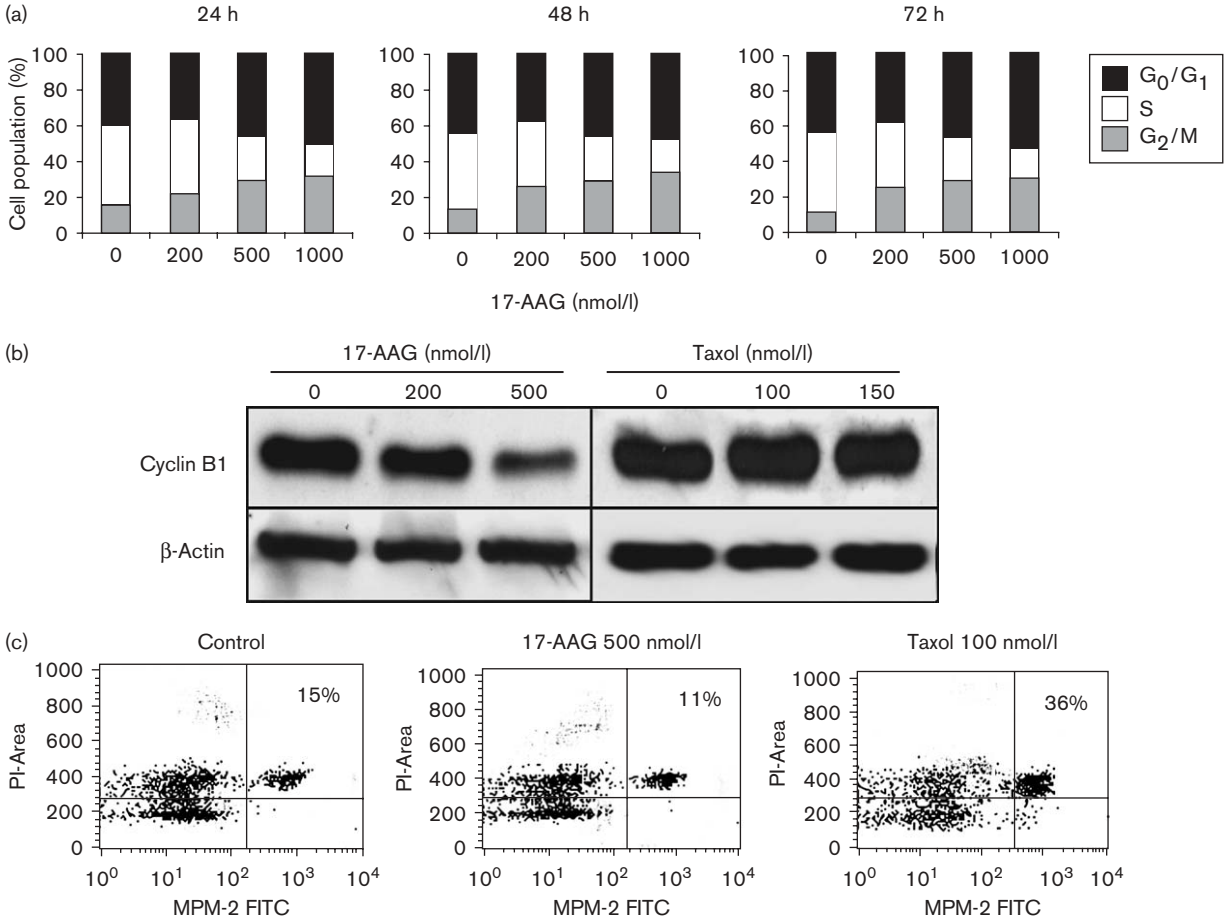


GL261 cells were treated with various concentrations of 17-allylamino-17-demethoxygeldanamycin (17-AAG) for up to 72 h. GL261 cell growth was inhibited in a dose- and time-dependent manner. Data are pooled from three independent experiments. Error bars indicate mean ± SD.

As flow cytometry does not distinguish cells in G_2 phase of the cell cycle from those that are in M phase, we performed additional experiments to assess the presence of mitotic cells. As a control, we used the known microtubule inhibitor taxol to induce M phase arrest. Representative data from two independent experiments are shown in Fig. 2b. First, we analyzed changes in the levels of expression of cyclin B1. Cyclin B1 forms a complex with the Cdc2 kinase that regulates entry of cells into G_2/M phase. Decreased levels of cyclin B1 can prevent progression of cells from G_2 into mitosis. Treatment of cells with 200 and 500 nmol/l 17-AAG resulted in a dose-dependent decrease in the levels of cyclin B1 expression, by 20 and 55%, respectively, compared with untreated cells. In contrast, the levels of cyclin B1 showed no change in cells treated with taxol (Fig. 2b).

To determine further whether 17-AAG-treated cells were arrested in G_2 phase versus M phase of the cell cycle, cells were analyzed for the expression of the mitosis-specific marker MPM-2 by flow cytometry [13,14]. Representative two-dimensional scatter plots of cells double positive for MPM-2 and 4*N* DNA content (PI staining) are shown from one of two independent experiments for 17-AAG-treated cells compared with

Fig. 2



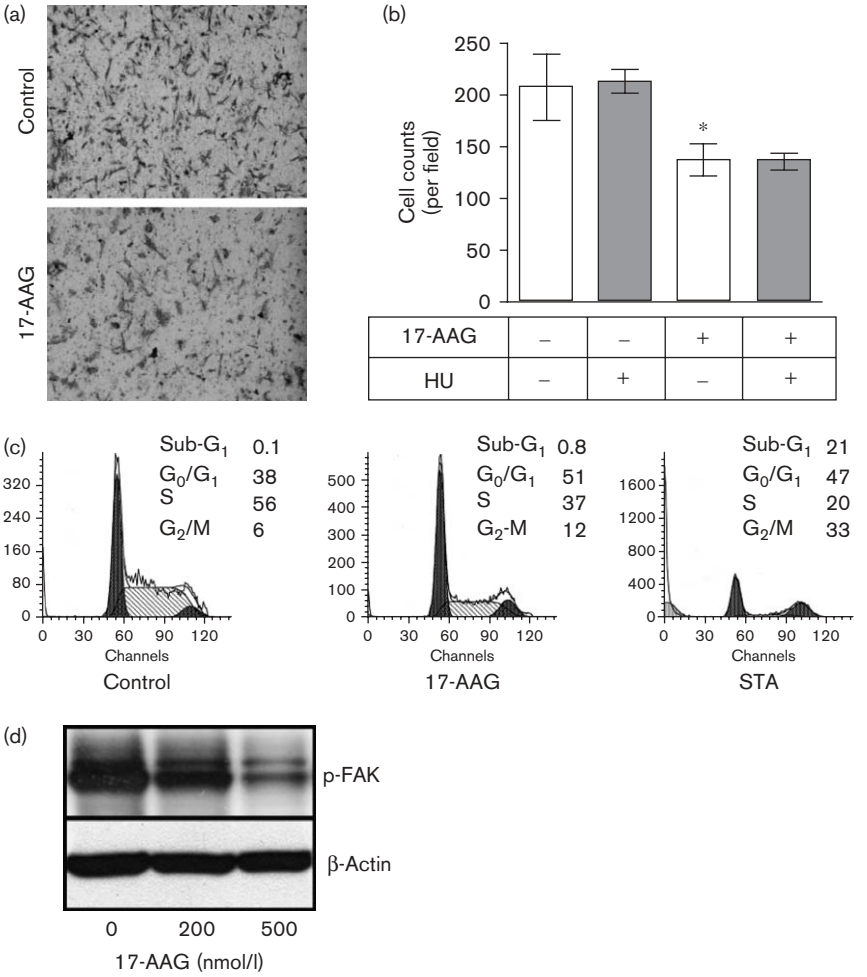
(a) Flow cytometry of cell cycle distribution in GL261 cells treated with various concentrations of 17-allylamino-17-demethoxygeldanamycin (17-AAG) for up to 72 h. GL261 cells showed growth arrest at the G_2/M phase of the cell cycle that was dose-dependent. Data are pooled from two independent experiments. (b) 17-AAG treatment resulted in a dose-dependent decrease in cyclin B1 expression, whereas no significant change in cyclin B1 expression was detected in taxol-treated cells. β-Actin was used as a loading control. (c) Cells were untreated or treated with 17-AAG or taxol and immunostained with the anti-MPM-2 monoclonal antibody to detect cells in M phase by flow cytometry. Only treatment with taxol, the M phase arresting agent, increased the number of MPM-2 positive cells.

taxol-treated cells (Fig. 2c). The percentage of MPM-2-positive cells was 11–15% in untreated and 17-AAG-treated cultures compared with 36% in cells treated with taxol that underwent M phase arrest. Taken together, these results are consistent with 17-AAG-induced G_2 phase arrest.

17-allylamino-17-demethoxygeldanamycin inhibits migration of GL261 cells

We tested the effect of 17-AAG on the ability of GL261 cells to migrate. After an incubation of 16 h in the absence or presence of 200 nmol/l 17-AAG, and in the absence or presence of 10 mmol/l HU, the numbers of migrated cells were quantified. Data from four independent experiments, with each assay performed in duplicate, are shown in Fig. 3. Photographs of one representative field from an insert containing control and drug-treated cells are shown (Fig. 3a). Bar graphs show the data pooled from the independent experiments (Fig. 3b). Migration of cells treated with 17-AAG for 16 h decreased by 43% compared with the control. This result was statistically significant ($P < 0.001$). The absence or presence of HU had no effect on cell migration. This result indicated that inhibition of cell proliferation was unlikely to contribute to the observed decrease in cell migration. To rule out the possibility that this difference in migration potential between drug-treated and non-treated cells was due to induction of cell death, we performed cell cycle analysis for the presence of the sub-G_1 peak that indicates cell fragmentation (Fig. 3c). STA was used as a positive control for induction of apoptosis of GL261 cells. Untreated cells or cells treated for 16 h with 200 nmol/l 17-AAG had a sub-G_1 peak less than 1%. In contrast, cells treated for 16 h with 1 μmol/l STA had a

Fig. 3



(a) GL261 cells were plated in migration chambers for 16 h in the absence or presence of 200 nmol/l 17-allylamino-17-demethoxygeldanamycin (17-AAG) and in the absence or presence of 10 mmol/l hydroxyurea (HU). An insert from a control or drug-treated culture shows the number of cells migrated after 16 h in one representative field. (b) Bar graphs represent data pooled from two independent experiments. Error bars indicate mean ± SD. *$P < 0.001$. (c) Flow cytometry of GL261 cells treated for 16 h with 200 nmol/l 17-AAG or 1 μmol/l staurosporine (STA) showed 1 versus 21% of apoptotic cells (sub-G_1 peak), respectively. (d) 17-AAG treatment resulted in a dose-dependent decrease in phospho-focal adhesion kinase (p-FAK) expression. β-actin was used as a loading control.

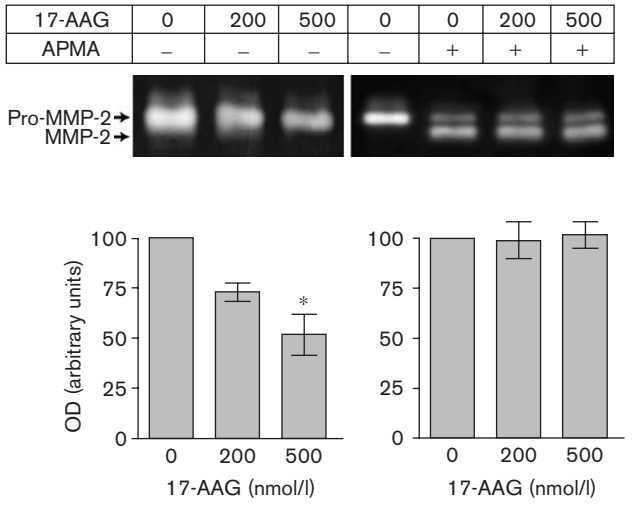
sub-G_1 peak greater than 20%. Thus, the decrease in migration detected in 17-AAG-treated cells at 16 h was not due to induction of cell death.

FAK, the initial protein to be tyrosine phosphorylated in the integrin signaling pathway, plays an important role in cell migration [17,18]. As we have shown previously that geldanamycin inhibited FAK phosphorylation in three human glioma lines concomitant with decreased cellular migration [2], we asked whether 17-AAG-induced inhibition of GL261 migration was also associated with decreased levels of FAK phosphorylation (p-FAK). Cells were treated with 200 and 500 nmol/l 17-AAG for 16 h and processed for Western blot analysis for p-FAK. The results of one representative experiment are shown in Fig. 3d. Treatment of GL261 cells with 17-AAG resulted in a dose-dependent decrease in the levels of p-FAK. These results taken together suggest that 17-AAG inhibits migration of GL261 cells, in part, by inhibiting phosphorylation of FAK.

17-allylamino-17-demethoxygeldanamycin inhibits metalloproteinase 2 secretion of GL261 cells

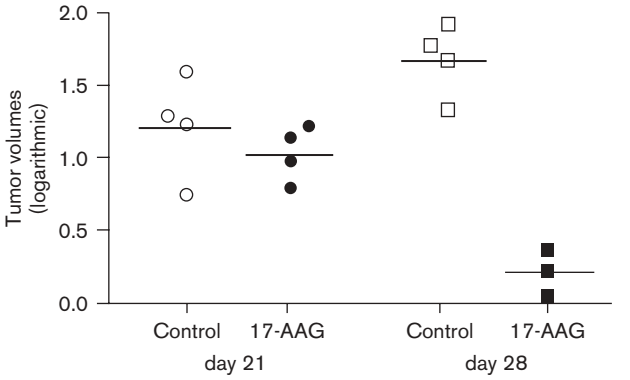
The secretion of proteolytic enzymes such as MMP-2 has been found to play a critical role in glioma cell migration and invasion [19,20]. MMP-2, a direct client protein of Hsp90, promotes the degradation of extracellular matrix proteins to create a permissive environment for glioma cell invasion. First, we determined the effect of 17-AAG treatment on MMP-2 secretion by GL261 glioma cells as shown in Fig. 4. Cells were incubated in the absence or presence of 200 and 500 nmol/l 17-AAG for 24 h and the

Fig. 4



GL261 cells were treated with 17-allylamino-17-demethoxygeldanamycin (17-AAG) for 24 h and the matrix metalloproteinase (MMP)-2 protein secreted into the culture medium was quantified by gelatin zymography. Conditioned media containing pro-MMP-2 was activated with *p*-aminophenylmercuric acetate (APMA) to produce active MMP-2 in the absence or presence of increasing concentrations of 17-AAG and the amount of MMP-2 was quantified by gelatin zymography. Bar graphs represent data pooled from three independent experiments. Error bars indicate mean ± SD. *$P < 0.001$.

Fig. 5



Effect of 17-allylamino-17-demethoxygeldanamycin (17-AAG) on tumor growth in C57BL/6 mice. Animals were inoculated intracranially with GL261 cells and allowed to grow for 7 days before 17-AAG treatment (closed symbols) or vehicle (open symbols) for 3 weeks. Tumor volumes were calculated at day 21 (circle) and day 28 (square), and are reported as log-transformed data. At day 21 the tumor volumes of the 17-AAG-treated animals showed no significant difference compared with untreated animals ($P = 0.38$). At day 28 the tumor volumes of the 17-AAG-treated animals were significantly smaller compared with untreated animals ($P = 0.002$, two-sided *t*-test). The horizontal lines represent the mean tumor volume for each group of animals.

conditioned media were analyzed for the amount of secreted MMP-2 using gelatin zymography. The results of one representative gelatin zymography experiment are shown together with the quantitative data pooled from three independent experiments. Secretion of MMP-2 by GL261 cells was decreased in a dose-dependent manner. The relative amount of MMP-2 secreted in cells treated with 500 nmol/l 17-AAG for 24 h compared with the untreated cells was reduced by 50%. This result was statistically significant ($P < 0.001$).

Next, we determined the effect of 17-AAG treatment on activation of MMP-2. MMP-2 is secreted by GL261 cells as the inactive zymogen pro-MMP-2 (72-kDa band) that can be activated extracellularly using the compound APMA to generate the active form of MMP-2 (66-kDa band) as shown in Fig. 4. To determine whether 17-AAG had inhibitory effects on MMP-2 activation, conditioned media containing pro-MMP-2 was untreated or treated with APMA to induce conversion of pro-MMP-2 to the activated MMP-2 form in the presence of increasing concentrations of 17-AAG. The results of one representative gelatin zymography experiment from three independent experiments are shown together with the quantitative data pooled from three independent experiments. Under these conditions, 17-AAG did not inhibit activation of MMP-2, because the 66-kDa bands remained the same intensity in the absence or presence of the drug.

17-allylamino-17-demethoxygeldanamycin exhibits anti-tumor effect on GL261 intracranial tumors

Intracranial tumors were established in C57BL/6 mice as described previously [10]. On day 7 after implantation, animals were randomly assigned into control and test groups, and treated with vehicle or 17-AAG (50 mg/kg) intraperitoneally three times a week for up to 28 days. Groups of animals were killed on days 21 and 28, respectively, and tumor volumes were measured. On average, animals treated with 17-AAG had smaller tumors at both days 21 and 28 compared with untreated animals as shown in Fig. 5. Often tumor volumes are distributed as log normal, typically with a few very large observations. Thus, it is common to report data after log transformation, as we have done here. At day 21 the tumor volumes of the 17-AAG-treated animals showed no significant difference compared with untreated animals ($P = 0.38$). At day 28 the tumor volumes of the 17-AAG-treated animals were significantly smaller compared with untreated animals ($P = 0.002$, two-sided *t*-test). As shown in Fig. 5, the log-transformed tumor volume data for each treatment day and each treatment group seem normally distributed (i.e. the data points are symmetrically distributed about the mean of each group). There appeared to be a trend up for increased tumor volumes for the control groups between days 21 and 28 ($P = 0.08$), as might be expected. There was a trend down for decreased tumor volumes for the 17-AAG-treated groups ($P = 0.0022$). Importantly, these results indicate that longer exposure of tumor-bearing animals to 17-AAG treatment produced greater inhibition of tumor growth.

Discussion

One of the major purposes of this study was to evaluate the anti-tumor effect of 17-AAG both *in vitro* and *in vivo* using our preclinical experimental GL261 murine model of glioma. As a class, the Hsp90 inhibitors possess potent anti-proliferative activity, usually at low nanomolar ranges on a wide variety of cultured cancer cell lines, including several human glioma cell lines [21–24]. Similarly in the murine GL261 glioma cell line tested in these studies, we found that 17-AAG at doses as low as 200 nmol/l showed anti-tumor activity within 24 h of treatment. One reason why Hsp90 inhibitors demonstrate such potent activity has recently been determined. The binding characteristics of geldanamycin were studied using purified Hsp90 protein. The results showed that the tight binding of geldanamycin to Hsp90 coupled with its very slow dissociation rate would promote accumulation of the drug within a cell, thus increasing its activity at much lower drug concentrations [21]. The amount of Hsp90 protein in normal cells comprises approximately 1–4% of the total cellular protein. As cancer cells overexpress Hsp90 protein, inhibitors of Hsp90 selectively accumulate within cancer cells compared with normal cells simply because of the law of mass action [21]. This pharmacological characteristic should also promote the accumulation of Hsp90 inhibitors *in vivo* in tumors over normal tissues and may account for the reasonable toxicity profile of 17-AAG in current clinical trials.

Hsp90 is a molecular chaperone required for the proper folding and maturation of many proteins, the so-called 'client protein substrates', involved in many cellular processes, including cell cycle regulation, that are commonly dysregulated in cancer cells. Thus, it is not surprising that Hsp90 inhibitors typically arrest cancer cells in G_1 and G_2/M phases of the cell cycle owing to the requirement for these client proteins to regulate cell cycle check points [25,26]. We showed that 17-AAG-treated murine GL261 glioma cells arrested in the G_2 phase of the cell cycle, based on combined results from flow cytometry and Western blot analyses, which was associated with the downregulation of cyclin B1. In contrast, our previous studies showed geldanamycin-treated human T98G glioma cells arrested in M phase, producing mitotic catastrophe [16]. Several studies have implicated the role of Hsp90 in the spindle checkpoint leading to arrest of cells in the G_2/M phase [25,26]. As Hsp90 chaperones a large number of client proteins, Hsp90 inhibitors are pleiotropic in their effects on cancer cells. The potential of Hsp90 inhibitors to act in a manner similar to microtubule inhibitors, much like the taxane class of anticancer drugs, may contribute to their selective action on cancer cells that are generally deficient in one or more of the cell cycle check points, compared with normal cells.

A feature of gliomas associated with their poor prognosis is the ability of tumor cells to invade the surrounding brain adjacent to the tumor that invariably gives rise to recurrent tumors. Recent evidence has demonstrated that Hsp90 serves as a chaperone for the extracellular matrix metalloproteinase MMP-2 [27,28]. In these studies, Hsp90 was shown to be necessary for the extracellular activation of MMP-2 required for invasion. Treatment of HT-1080 fibrosarcoma cells with geldanamycin not only inhibited the activation and secretion of MMP-2 into the medium but also blocked invasion of the cells through a membrane [28]. Given this association already established between geldanamycin, MMP-2 and invasion, we wished to test whether the 17-AAG analogue would similarly affect migration of GL261 glioma cells and secretion of MMP-2. Our results showed that low doses of 17-AAG significantly inhibited migration within 16 h of treatment concomitant with inhibition of MMP-2 secretion. Similar to our studies in human glioma cell lines where geldanamycin inhibited migration and phosphorylation of FAK [2], 17-AAG treatment of the murine GL261 glioma cell line also decreased levels of phosphorylated FAK.

The geldanamycin analogues have shown anti-tumor activity in several animal models including breast, colon, melanoma, neuroblastoma, ovarian, pancreatic, prostate and small-cell lung cancers [29–34]. For glioma, only one report has shown efficacy of 17-AAG in a xenograft model using the C6 rat glioma cell line grown subcutaneously in nude mice [35]. In this study, we have used an orthotopic glioma model with well-established intracranial tumors to test the anti-tumor efficacy of 17-AAG. When tumor-bearing animals were treated with 3 weekly cycles of 17-AAG, there was a significant reduction of tumor volumes compared with untreated control animals. Taken together our data provide a strong rationale for use of geldanamycin analogues, such as 17-AAG, in the treatment of patients with gliomas that are highly invasive and refractory neoplasms.

In conclusion, the Hsp90 inhibitors represent a highly promising class of anticancer drugs [3–7]. They were recently cited as one among many promising approaches currently being considered for treatment of glioma [36]. As the 17-AAG analogue is currently in phase I/II clinical trials at numerous institutions for a wide range of cancer types, an effort has been made to identify relevant biomarkers that can readily follow the anti-tumor activity of Hsp90 drugs in the serum of patients [37]. Insulin-like growth factor binding protein-2 (IGFBP-2) is elevated in the serum of cancer patients and has been shown to correlate positively with tumor burden and tumor recurrence [37]. Mice bearing human xenografts that were treated with 17-AAG demonstrated decreased levels of IGFBP-2 in the serum that correlated with inhibition of Hsp90 activity [37]. Serum levels of IGFBP-2 were significantly elevated in breast and brain tumor patients compared with prostate and melanoma patients [37]. Thus, detection of decreased levels of IGFBP-2 in serum

could be used for monitoring response of glioma patients treated with Hsp90 inhibitors. Given that 17-AAG significantly decreased the growth of GL261 glioma *in vivo*, clinical testing of 17-AAG or other novel Hsp90 inhibitors being developed should be considered for glioma patients whose tumors remain refractory to most current treatment regimens.

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